## ÁREA PECUARIA

# NUTRITIONAL CHARACTERIZATION OF RAW MATERIALS WITH ALTERNATIVE APPLICATIONS FOR THE FEEDING OF Oreochromis Niloticus

CARACTERIZACIÓN NUTRICIONAL DE MATERIAS PRIMAS COMO ALTERNATIVA DE USO PARA LA ALIMENTACIÓN DE *Oreochromis niloticus* 



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Resumen: Contextualización: La actividad piscícola ha crecido rápidamente en Colombia, representando el 3.3% del PIB agrícola, con énfasis en la pesca continental de especies como *Oreochromis niloticus, Colossoma macropomum y Oncorhynchus mykiss.* Este crecimiento impulsa la demanda de alimentos balanceados y la búsqueda de ingredientes alternativos para mejorar la rentabilidad de las piscifactorías mediante dietas eficientes y sostenibles.

Principio del formulario

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Vacío de conocimiento: El crecimiento constante y acelerado de la producción piscícola a nivel nacional y departamental, ha aumentado la demanda de alimentos balanceados, influyendo en los costos de producción que pueden ser hasta del 70% del total, haciéndose necesaria la búsqueda de productos que logren cubrir las necesidades nutricionales de los peces por medio de la inclusión de ingredientes alternativos.

**Propósito:** Determinar la composición nutricional de 14 materias primas alternativas entre materiales vegetales y subproductos agrícolas, presentes en la Provincia de Lengupá, para la alimentación de Tilapia.

Metodología: El estudio se realizó en la Provincia de Lengupá, abarcando 1645 km², con municipios como Miraflores, Páez, Campohermoso, Zetaquira, Berbeo, San Eduardo y Rondón, en donde se seleccionaron 14 materias primas entre materiales vegetales y subproductos agrícolas (Manihot esculenta, Colocasia esculenta, Morus spp, Sambucus nigra, Tithonia diversifolia, Arracacia xanthorrhiza, Arachis pintoi, Plukenetia volubilis, Theobroma cacao, Coffea arabica Glycine max, Zea mays, Palm Kernel, Oryza sativa), a las cuales se les evaluó su composición nutricional, mediante análisis bromatológico, determinando el contenido de Humedad, Materia Seca, Ceniza, Extracto Etéreo, Proteína Cruda, Fibra Detergente Ácido, Fibra Detergente Neutro, Energía Bruta, Calcio y Fósforo.

Resultados y conclusiones: Se encontraron variaciones en el contenido de fibra, proteína, grasas, minerales y energía en las especies vegetales, siendo *Manihot esculenta* la de mayor contenido proteico (22.66%) y energético (4574 cal/g), por lo que podría ser considerada para reducir las fuentes tradicionales en la formulación de dietas piscícolas. Dentro de los subproductos agrícolas, *Glycine max* se destacó como líder en proteína (46.33%) y la torta de *Plukenetia volubilis* como una fuente importante de energía (5386.6 cal/g). Por lo que las fuentes evaluadas podrían emplearse en la formulación de dietas que cumplan con los requerimientos nutricionales de la tilapia, considerando aspectos como la digestibilidad y equilibrio de nutrientes.

Palabras clave: *Manihot esculenta*, *Glycine max*, Piscicultura, Subproductos, Tilapia.

Abstract: Contextualization: Fish farming has rapidly grown in Colombia, representing 3.3% of the agricultural GDP, with an emphasis on freshwater fishing of species such as *Oreochromis niloticus, Colossoma macropomum y Oncorhynchus mykiss*. This growth drives the demand for balanced food and the search for alternative ingredients to enhance the profitability of fish farms through efficient and sustainable diets.

Knowledge gap: The steady and rapid growth of fish production at the national and departmental levels has increased the demand for balanced food, impacting production costs, which can account for up to 70% of the total. This necessitates the search for products that can meet the nutritional needs of fish through the inclusion of alternative ingredients.

**Purpose:** Analyze the nutritional composition of 14 alternative raw materials including plant materials and byproducts, present in the Lengupá Province, for Tilapia feeding.

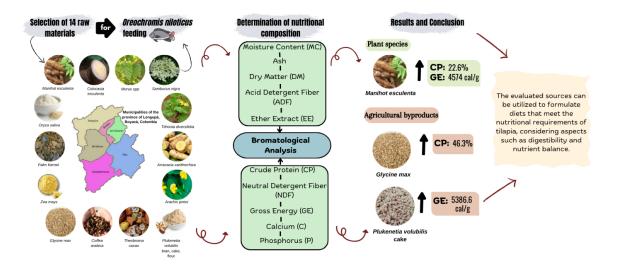
Methodology: The study was conducted in the Lengupá Province, covering an area of 1645 km², including municipalities such as Miraflores, Páez, Campohermoso, Zetaquira, Berbeo, San Eduardo, and Rondón. Fourteen raw materials (Manihot esculenta, Colocasia esculenta, Morus spp, Sambucus nigra, Tithonia diversifolia, Arracacia xanthorrhiza, Arachis pintoi, Plukenetia volubilis, Theobroma cacao, Coffea arabica, Glycine max, Zea mays, Palm Kernel, Oryza sativa) were selected for nutritional composition analysis through bromatological analysis, determining moisture content, dry matter, ash, ether extract, crude protein, acid detergent fiber, neutral detergent fiber, gross energy, calcium, and phosphorus.

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Results and conclusions: Variations were found in fiber, protein, fat, minerals, and energy content among the plant species, with Manihot esculenta having the highest protein content (22.66%) and energy (4574 cal/g). Thus, it could be considered to reduce traditional sources in fish diet formulation. Agricultural by-products were also compared, with Glycine max standing out as a protein leader (46.33%) and Plukenetia volubilis cake as an important energy source (5386.6 cal/g). Therefore, the evaluated sources could be used in formulating diets meeting the nutritional requirements of tilapia, considering aspects such as digestibility and nutrient balance.

**Keywords:** By-products, energy, food, protein, tilapia, vegetable.

### **GRAPHIC SUMMARY**



Representative Scheme of Methodology, Results, and Conclusions of the Article

#### 1. INTRODUCTION

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Aquaculture stands as one of the fastest-growing livestock activities in Colombia, contributing significantly with a production of 174,067 tons per year and constituting 3.3% of the agricultural GDP (Pineda-Santis et al., 2023). These activities can be broadly categorized into three main groups: marine fishing (21.3%), inland fishing (3.3%), and predominantly, freshwater continental aquaculture (75.4%). The latter is composed of nilotica and red tilapia (58%), white and black cachama (19%), trout (16%), and some native species (7%). It is noteworthy that this endeavor is undertaken by artisanal fishermen and farmers, representing nearly one-third of the combined national production from capture and aquaculture. This surpasses the average growth rate of the agricultural sector and the overall national economy (Zapata, 2020).

Between 2015 and 2020, the production in the Boyacá department incremented from 1784 to 4482 tons, encompassing red and silver tilapia, trout, and cachama, distributed across various subregions of the department (Zapata, 2020). This consistent and accelerated growth in national and departmental production has exerted pressure on the demand for balanced feed intended for these species, directly influencing production costs that can fluctuate between 50% and 70% of the total variable production expenses (Solarte et al., 2022).

Corbalá-Bermejo et al. (2019), relate the value of fish feeds to its ingredients with fishmeal being the most expensive. Vegetable-based meals, such as corn and soybean meal, directly compete with human consumption (Castillo-Luna et al., 2022). Due to the socio-economic dynamics in recent years, all countries are struggling with global price variations, presenting significant challenges to the productive sector. Consequently, the aquaculture food industry has been exploring products that meet the nutritional needs of fish while simultaneously increasing the inclusion of alternative ingredients (Corbalá-Bermejo et al., 2019).

Given that the quality and cost of feed are critical factors for the profitability of a fish farm (Barragán et al., 2017), it is imperative to assess diets that aim to be more efficient and of high quality, improving productivity and animal health without negatively impacting the environment or inflating production costs. Balanced rations must encompass all the necessary nutrients for fish to grow at a desirable rate, reproduce adequately, and maintain good health throughout their development. Hence, the need to evaluate raw materials that can cover these nutritional requirements, considering that deficiencies or excesses of nutrients and characteristics like fiber content can adversely affect animal productivity and increase waste excretion into the environment (Pineda-Santis., et al 2023).

To identify sustainable and cost-effective alternatives to produce balanced aquaculture feeds, the nutritional composition and nutrient availability of 14 alternative raw materials between plant material and agricultural byproducts were evaluated. This serves as an initial step to determine their viability as ingredients in the formulation of well-balanced and healthy fish diets.

### 2. MATERIALS AND METHODS

The current study was conducted in the Lengupá Province, situated in the Eastern Cordillera, spanning 1645 km² from the Bijagüal paramo to the foothills of the Llanero piedmont, the area presents a diverse distribution of biodiversity, with ecosystems ranging from 300 to 3600 meters above sea level (masl). This region includes municipalities such as Miraflores, Paéz, Campohermoso, Zetaquira, Berbeo, San Eduardo and Rondón. In Miraflores raw materials such as *Manihot esculenta* (5°10′59.6″N, 73°09′32.6″W), *Colocasia esculenta* (5°10′59.6″N, 73°09′32.6″W), *Sambucus nigra* (5°10′59.6″N, 73°09′32.6″W), *Arachis pintoi* (5°10′59.6″N, 73°09′32.6″W), *Plukenetia vaolubilis* (5° 9′ 59.271″ N, 73° 10′ 42.695″ W), *Theobroma cacao* and *Coffea arabica* (5° 12′ 5.588″ N, 73°, 8′ 19.028″ W) were collected. *Morus alba* and *Arracacia xantorrhiza* were collected in San Eduardo (5° 12′ 38.412″ N, 73° 5′ 56.2″ W), while in the municipality of Berbeo *Tihtonia diversifolia* was collected (5° 13′ 44.459″ N, 73° 8′ 8.992″ W). The raw materials *Glycine max*, *Zea mays*, *Palm kernel* and *Oryza sativa* were purchased commercially from local supermarkets.

### Sampling

Fourteen alternative and conventional raw materials, present within the municipalities of the Lengupá Province, were selected for this study (Table 1). Sample selection took place within productive properties, and to ensure a significant sample, the area where the species of interest were present was traversed in an X or Z pattern. Plants with a healthy appearance were specifically chosen for sampling in the required tissues. For the sampling of by-products, visits were made to specialized producers, obtaining 500g sub-samples of fresh by-products, which were later combined to obtain representative samples (Figure 1). Material transportation was carried out on the same day of collection using airtight sealed bags and paper towels to reduce moisture and prevent fungal proliferation. Finally, the samples were transported in coolers to the laboratory.

Table 1. Selected Raw Materials for Proximate Analysis.

Plant Species	Tissue	By- Products	Presentation	Traditional Materials	Presentation	
Cassava Manihot esculenta	Leaf		Bran	Soybean Glycine max	Cake	
Bore <i>Colocasia</i> <i>esculenta</i>	Stem	Sacha Inchi Plukenetia volubilis	Cake	Corn	Flour	
Mulberry <i>Morus spp.</i>	Leaf		Flour	– Zea mays		
Elderberry Sambucus nigra	Leaf	Cocoa				
Shrub sunflower Tithonia diversifolia	Leaf	Theobroma cacao	Cacota	Palm Kernel	Cake	
Arracacha <i>Arracacia</i> xanthorrhiza	Tuber	Coffee <i>Coffea</i>	Cacota	Rice	Flour	
Pinto peanut, Arachis pintoi	Leaf	arabica		Oryza sativa		

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**Figure 1.** Raw materials used in the study.

A)Tithonia diversifolia; B) Oryza sativa; C) Zea mays; D) Arracacia xantorrihza; E) Morus alba; F) Coffea arabiga; G) Plukenetia vaolubilis; H)
Manihot esculenta; I) Colocasia esculenta; J) Arachis pintoi; K) Sambucus nigra; L) Theobroma cacao; M) Glycine max.

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### **Nutritional Composition Analysis:**

The collected samples were taken to the Animal Nutrition Laboratory of the Pedagogical and Technological University of Colombia, located in the city of Tunja, Boyacá. The bromatological composition of the raw materials was analyzed following the methodology described by the Association of Analytical Communities -A.O.A.C- (AOAC, 2019). The chemical composition of the collected samples was determined in triplicate as described below:

- 1. Dry Matter (DM) and Moisture (H): The sample was dried in a MEMMERT® forced ventilation oven at 60°C for 48 hours.
- 2. Crude Protein (CP): Analyzed from total nitrogen using the Kjeldahl method, leading to digestion of 0.2. g of sample with selenium, plus 7 mL of 97% H<sub>2</sub>SO<sub>4</sub>, using analytical grade MERCK \* reagents.
- 3. Ash (CZ): Determined by incinerating dry matter at 550°C for 4 hours in a muffle furnace MEMMERT\*.
- 4. Crude Fiber (FC %): The defatted sample was subjected to boiling solutions of sulfuric acid and potassium hydroxide, washed, dried, weighed, and then incinerated at 500°C, using analytical grade MERCK \* reagents.

For energy analysis through sample combustion in a calorimeter bomb and determination of mineral content, specifically calcium and phosphorus, through the decomposition of plant material, the following reagents were used: Linde brand oxygen  $(O_2)$  for combustion in the calorimeter bomb, MERCK  $^{\circ}$  nitric acid  $(HNO_3)$  and hydrogen peroxide  $(H_2O_2)$  for sample decomposition and digestion, and MERCK  $^{\circ}$  or Sigma-Aldrich  $^{\circ}$  standard calcium and phosphorus solutions as a reference for mineral analysis. These analyses were carried out at the Chemical and Bromatological Analysis Laboratory of the National University of Colombia (UNAL) in the Medellín campus.

### Statistical Analysis

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The values obtained for each of the variables, assessed in triplicate, were analyzed using a one-way analysis of variance (ANOVA) with Tukey's test to determine whether there were significant differences (p-value ≤ 0.05). The Shapiro-Wilk and Bartlett tests were employed to check the assumptions of normal distribution and homogeneity of variance. These analyses were performed using the agricolae package in the R Studio statistical software, version 7.1 (R Core Team, 2020).

### 3. RESULTS AND DISCUSSION

The utilization of alternative raw materials must be carried out in a way that the formulated diet is balanced to cover the necessary nutritional requirements for fish, enabling them to perform normal functions such as tissue development. Additionally, these materials should serve as a source of energy for other functions like nutrient absorption throughout their normal life cycle (Bittencourt et al., 2018; Eriegha and Ekokotu, 2017). Therefore, a balance must exist between two key components of diets, namely energy and protein. An excess of energy in relation to the amount of protein in the diet may result in elevated fat deposition, reduced feed intake, and decreased weight gain in animals. On the contrary, an excess of protein can lead the animal to use protein amino acids as a source of energy, expelling excess NH<sub>3</sub>, thus increasing greenhouse gas emissions (Botello-León et al., 2022).

Seven (7) plant species and five (5) by-products were selected as alternative raw materials for use as ingredients in the formulation of aquaculture diets. Conventional raw materials commonly used in diet formulation were also analyzed compositionally for comparison against the alternative raw materials.

## Plant Species

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The nutritional content of *cassava leaves*, mulberry leaves, elderberry leaves, Shrub sunflower leaves, and Pinto peanut leaves were analyzed, while the root of arracacha and the stem of bore were analyzed (Table 2). Variations were observed in terms of fiber, protein, fats, minerals, and energy content, which could contribute to the diets for feeding tilapia and cachama. According to Eriegha & Ekokotu. (2017), and Bittencourt et al. (2018), the use of alternative raw materials should meet the necessary nutritional requirements for fish to facilitate tissue development and serve as an energy source for other functions in their normal life cycle. Therefore, a balance between energy and protein is crucial (Manam, 2023).

According to the review conducted by Engdaw y Geremew (2024), regarding the reported nutritional requirements for *Oreochromis niloticus*, some of the evaluated species may be suitable for their feeding, particularly in terms of protein and phosphorus.

Table 2. Proximate Analysis of Plant Species.

Species	Cassava	Bore	Mulberry	Elderberry	Shrub sunflower	Arracacha	Pinto peanut	P- Value
Tissue	Leaf	Stem	Leaf	Leaf	Leaf	Tuber	Leaf	value
Moisture (%)	8.60e	5.50f	9.83d	14.33b	12.67c	16.16ª	8.33e	<0.001
Dry Matter (%)	91.46abc	94.83a	90.26bc	85.73d	87.23cd	84.00d	91.76ab	<0.001
Ash (%)	8.60d	9.83c	8.56d	1.76f	12.90b	4.16e	15.83a	<0.001
Crude Fat (%)	5.56a	1.10d	0.80d	1.80c	4.33b	0.83d	1.63cd	<0.001
NDF (%)	33.8d	48.25b	37.9c	47.3b	24e	7.25f	54a	<0.001
ADF (%)	27.5c	36.4b	29.5c	30c	19.6d	6.3f	40a	<0.001
Crude Protein (%)	22.66a	5.90d	14.63c	14.17c	20.00b	3.96e	20.16b	<0.001
Calcium g*kg <sup>-1</sup>	2.00ab	0.13d	1.73b	1.83b	1.96ab	0.93c	2.40a	<0.001
Phosphorus g*100g <sup>-1</sup>	0.32b	0.32b	0.31b	0.32b	0.30b	0.23b	0.54a	<0.007
Gross Energy cal*g <sup>-1</sup>	4574a	3262d	3944c	4386	3966c	3406d	3882c	<0.001

H = Moisture; MS = Dry Matter; CZ = Ash; PC = Crude Protein; FC = Crude Fiber; EE = Ether extract (Crude Fat); FDN = Neutral Detergent Fiber; FDA = Acid Detergent Fiber.

Means with different letters within the same row differ significantly from each other, according to the Tukey test  $(p \le 0.05)$ .

It is important to consider not only the direct aspects of nutritional composition, but also the environmental and management factors that influence the quality of these materials (Fonseca et al., 2024). Effective management practices such as soil fertility and fertilizer use play a crucial role in optimizing nutrient levels, in addition, climatic conditions, such as temperature, precipitation and sunlight, can significantly affect nutrient uptake and assimilation by plants. Other factors, such as the presence of pests or diseases and soil quality, can also contribute to overall nutrient content in plant tissues. Therefore, a comprehensive understanding of these variables is essential to ensure optimal nutrient concentration in plants (Fonseca-López et al., 2024).

### Protein Content and Energy

Regarding protein content, the optimal range reported for *Oreochromis niloticus* is between 26.8% and 41.3%. According to the results, cassava shows the highest crude protein content (22.66%). Although it is outside the optimal range, it could be considered if combined with other protein sources in the diet formulation. Studies by Pawhestri et al. (2020), showed that individuals fed with a mix of 75% fish meal and 25% cassava leaf meal had higher weight gain compared to conventional feed.

For Shrub sunflower, a protein content of 20% was found, lower than that reported by Pineda-Santis et al. (2023), who used 20% Shrub sunflower as a dietary supplement for Oreochromis sp. and observed improved zootechnical parameters compared to commercial diets. However, crude protein contents for elderberry (14.17%) and mulberry (14.63%) did not differ significantly, showing lower protein contents compared to conventional protein sources like soybean meal, poultry and fish meals. These values are also lower than those reported by da Silva Dias et al. (2022), who obtained a crude protein content of 24.45% for mulberry, demonstrating its positive impact on fish growth, antioxidant activity, and immune response in *O. niloticus*.

Regarding raw energy, cassava has a high content of (4574 calg<sup>-1</sup>), which makes it a potential source to meet the energy requirements of *O. niloticus*. A study by Pawhestri et al. (2020) showed that a 25% inclusion of cassava flour in the diet resulted in optimal growth. However, it is important to consider that the actual digestible energy of tilapia may be slightly lower, around 70% of raw energy consumed (Engdaw & Geremew, 2024). Although, cassava remains a promising option in terms of energy. Other species such as bore, mulberry, elderberry, shrub sunflower and arracacha have raw energy values in the intermediate range (between 3406 and 4386 calg<sup>-1</sup>) (Engdaw & Geremew, 2024), suggesting the need to supplement with more energy rich sources, especially during growth phases.

In contrast, Pinto peanuts have significantly lower gross energy content (3262 cal\*g<sup>-1</sup>), which could provide less energy than recommended for the various tilapia growth stages Engdaw y y Geremew, 2024; Lall y Kaushik, 2021). The digestibility of raw materials is crucial in the formulation of tilapia diets, as it directly affects the absorption of nutrients and the growth of the tilapia. Although cassava has a high raw energy content, its digestibility may be compromised due to its high fiber content (33% NDF), which could reduce the actual energy availability for tilapia.

### Fiber Content

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The fiber content is crucial, an excessive dietary fiber can lead to gastrointestinal disturbances in these monogastric fish. It is important to classify raw materials as either promising or not, based on their crude fiber content. A comprehensive evaluation of raw materials for feeding is essential, and relying solely on

crude fiber content may result in insufficient data. The analysis should consider neutral detergent fiber (NDF) and acid detergent fiber (ADF) content, as these values provide crucial information about the digestible fraction of foods. Low NDF content is generally associated with a higher content of cellular components (CC), contributing to a higher nutritional value. However, it's important to note that fibrous foods significantly impact the digestion of the lipid fraction in monogastric animals (Botello-León et al., 2020; Botello-León et al., 2022).

Average NDF values of 24% and 33% were observed for Shrub sunflower and cassava, respectively. These values align with those reported by Puerta Rico et al., (2017), who indicated that Shrub sunflower had a low-medium fiber content, mainly represented by lower levels of cellulose and lignin (ADF), which are associated with higher digestibility. However, higher NDF and ADF values, as seen in bore, elderberry, mulberry, and Pinto peanut, could be linked to lower diet digestibility by *P. brachypomus*. According to the classification standards proposed by the American Forage and Grassland Council (Botello-León et al., 2020), except for bore, the evaluated plant species have an NDF lower than 41%, suggesting they could be classified as forages of excellent quality.

#### Arracacha as a Source

Arracacha tuber presented an NDF content of 7.2%, in line with values reported by Luziatelli et al. (2023). They highlighted arracacha as a good source of ascorbic acid (vitamin C), vitamin A, and minerals, especially calcium (0.93g\*kg<sup>-1</sup> in this study). They also reported protein contents below 4% of DM, like this study, suggesting that although arracacha may be valuable due to its low fiber content and being a good source of vitamins and minerals, it is not a significant protein source.

According to the obtained results, soybean meal leads in terms of crude protein, calcium, and phosphorus content. Additionally, it showed a content of 4194.3 cal/g. While soybean meal has been strongly recommended by various studies (González et al., 2014; Sánchez et al., 2020), it has been demonstrated that formulating diets with soybean as a protein and energy source can negatively impact the weight gain of O. niloticus when used in values exceeding 40% (Barragán et al., 2017; Obirikorang et al., 2020).

### Byproducts

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The nutritional content of soybean, corn, palm, kernel, rice, sacha inchi, cocoa and coffee was analyzed as shown in table 3. Comparing these food sources with the previously evaluated plant species, it is observed that, in general, animal protein sources have a relatively lower protein content compared to the mentioned plant protein sources such as Cacao Husk and plant species like Cassava. Although soybean meal led in protein content, sacha inchi cake presented 31.80% of crude protein, being an important source to meet nutritional requirements for the reversal phases of *O. niloticus*, calculated at 29.73 and 26.8%. Studies related with sacha inchi have shown that the unprocessed cake can have a digestibility of over 80% in tilapia (Henao and Barreto, 2021), with protein contents of 41.49% (Alcívar et al., 2020). On the other hand, coffee presented a crude protein content of 23.63%, exceeding the value obtained by Oropeza-Mariano et al. (2022), who evaluated the inclusion of coffee pulp in the diet of fry, demonstrating that inclusion up to 20% improved the weight gain of fry compared to the control diet.

Table 3. Proximate Analysis of By-Products

Material	Soybean	Corn	Palm Kernel	Rice		Sacha Inchi		Cocoa	Coffee	P-
Presentation	Cake	Flour	Cake	Flour	Bran	Cake	Flour	Husk	Husk	Value
Moisture (%)	6.90f	8.13e	8.86de	10.56c	6.36fg	5.90g	9.33d	14.66a	11.80b	<0.001
Dry Matter (%)	93.10b	91.86c	91.13cd	89.43e	93.63ab	94.10a	90.66d	85.33g	88.20f	<0.001
Ash (%)	6.63b	5.26cd	3.86ef	2.33g	3.40f	3.86ef	4.30d	7.96a	6.13bc	<0.001
Crude Fat (%)	1.16g	3.33e	7.93c	0.46h	24.13a	9.23b	4.30d	2.43f	3.93de	<0.001
Crude Protein (%)	6.90e	2.96h	27.33c	1.46i	22.40d	5.63f	4.23g	28.53b	36.13a	<0.001
Crude Fiber (%)	46.33a	8.83f	14.53e	7.20g	18.60d	31.80b	23.63c	7.56g	4.36h	<0.001
Calcium (g*kg <sup>-1</sup> )	0.333ab	0.178b	*	0.133b	*	*	0.333ab	*	0.566a	<0.001
Phosphorus (g*100g <sup>-1</sup> )	0.666a	0.296b	*	0.146b	*	*	0.490ab	*	0.183b	<0.001
Gross Energy (cal*g <sup>-1</sup> )	4194.3b	3920.0c	*	3704.6d	*	*	5386.6ª	*	3950.3c	<0.001

H = Moisture; MS = Dry Matter; CZ = Ash; PC = Crude Protein; FC = Crude Fiber; EE = Ether Extract (Fat); FDN = Neutral Detergent Fiber; FDA = Acid Detergent Fiber.

Means with different letters within the same row differ from each other, according to the Tukey test  $(p \le 0.05)$ .\* Corresponds to values not calculated in this study.

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In terms of gross energy content, coffee (5386.6 cal\*g<sup>-1</sup>) and soybean meal (4194.3 cal\*g<sup>-1</sup>) are the highest-energy raw materials, making them an interesting option as an energy source for tilapia. However, despite maize, broken rice, and coffee having lower energy content, they were above the energy recommendations reported by Engdaw & Geremew. (2024), who reported 4007, 3036, and 3075 cal\*kg<sup>-1</sup> of Digestible Energy. This coincides with what was reported by Phinrub et al. (2024), who found that it is posible to replace up to 15% of the energy sources in the diet of the Nile tilapia with coffee husks without significantly altering the biochemical serum indices of the fish.

Regarding phosphorus content, the estimated range for feeding *O. niloticus* is from 0.75% to 0.46% (Eriegha 2017). Pinto peanut has the highest phosphorus content (0.54 g\*100g<sup>-1</sup>), suggesting that it could be suitable to meet the phosphorus requirements of *O. niloticus*. It is important to note that the phosphorus and calcium content is also crucial in the tilapia diet to maintain a balanced P/Ca ratio and avoid water contamination issues. In this regard, the evaluated food sources seem to offer adequate levels of these minerals, although the specific values vary among sources. The ratio between these two elements is essential for the formation of bone hydroxyapatite (Lall and Kaushik and 2021). Similarly, according to the results of Hisano et al. (2023), the availability of phosphorus and calcium positively influences the intestinal morphology of *O. niloticus* juveniles.

The determination of statistically significant differences in the nutritional content of the raw materials studied, highlights the importance of taking advantage of them as a potential source of essential nutrients, since this diversity in nutritional profiles can allow the formulation of balanced feeds that are specifically adjusted to the needs of each production stage of *Oreochromis niloticus*. By combining these alternative feeds, a diet can be designed that not only optimizes the growth and health of the fish, but also reduces dependence on traditional ingredients commonly used in the production of aquaculture feeds, which diversifies the sources of inputs, contributing to the sustainability of the sector, offering viable alternatives in regions with limited access to conventional raw materials.

Ingredient digestibility is crucial in tilapia diet formulation, as it affects feed conversion efficiency and fish growth. Arracacha, with a low fiber content (7.2% NDF), has high digestibility, which is beneficial for tilapia as it allows for better nutrient absorption (Luziatelli et al., 2023), this being especially useful during the initial growth phases, when fish require a diet that facilitates the rapid absorption of essential nutrients. In contrast, soybean meal, although an excellent source of protein and energy, can have negative effects if used in high proportions due to its potential impact on digestion and gut health in fish (Howlader et al., 2023).

It has been shown that the excessive inclusion of soybean meal can lead to lower feed conversion efficiency and digestive health problems in tilapia, however, it remains one of the most widely used sources of vegetable protein in aquaculture due to its high protein content, balanced amino acid profile, wide availability and low cost. In addition to its contribution to sustainability by reducing dependence on fishmeal, the key is to use it in a balanced way, combining it with other nutrient sources to maximize benefits and minimize potential negative effects. (Gule & Geremew, 2022).

Coffee and sacha inchi are ingredients with high protein and energy content, which makes them attractive for the formulation of fish farming diets. Sacha inchi, with a digestibility of more than 80% in tilapia, is presented as an excellent candidate to provide high-quality proteins (Khieokhajonkhet et al., 2021). On the other hand, coffee, although it has a high energy content (5386.6 cal/g), can present challenges in terms of digestibility, so its inclusion must be carefully balanced to avoid digestive problems (Engdaw and Geremew, 2024).

According to Alcívar (2020), the formulation of balanced feeds must take into account raw materials that meet the nutritional requirements of tilapia, reaching estimated crude protein contents of at least 24% and crude energy of 3600 kcal/kg, the following formulation is suggested for a balanced feed, including the ingredients presented in table 4, which was developed in the research project from which this scientific article is derived.

Table 4. Suggested comercial formula for a supplementary diet for the finishing stage of tilapias based on a 24% crude protein

RAW MATERIAL	% INCLUSION	gr/kg	
Glycine max flour	32.5	325	
Zea mayz flour	16.7	167	
<i>Morus alba</i> leaf	10.0	100	
Tithonia diversifolia leaf	9.9	99.3	
Arachis pintoi leaf	5.0	50.0	
Plukenetia vaolubilis	5.0	50	
Coffea arabica Cacota	5.0	50	
Theobroma cacao Cacota	5.0	50	
Arracacia macrorrhiza	3.0	30	
Oryza sativa flour	2.0	20	
Vegetable oil	4.0	40	
Molasses	1.0	10	
Vitamins + Minerals Premix	0.3	2.5	
Salt or sea salt	0.3	2.5	
Choline chloride	0.1	1.0	
Calcium carbonate	0.1	1.0	
Dicalcium phosphate	0.1	1.0	
Rovimix Vitamin C	0.1	0.5	
Antioxidant - BTH	0.01	0.3	
TOTAL	100	1000	

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Among the materials studied, soybean meal is a primary source of vegetable protein with an amino acid profile that complements the tilapia diet. Although it has antinutritional factors such as trypsin inhibitors, its balanced inclusion maximizes protein absorption, improving growth and feed conversion. It's true protein digestibility has been reported to be between 90% and 95% in tilapia (Magbanua & Ragaza, 2024).

The inclusion of *Zea mayz* flour provides energy to the diet in the form of highly digestible carbohydrates, with an apparent starch digestibility of more than 85% (Hossain et al., 2023). Its inclusion in the diet balances the energy content, promoting efficient growth without altering protein digestibility, while *Morus alba* leaves have a high protein and mineral content, and provides bioactive compounds that improve intestinal health and immunity in tilapia (Días et al., 2022). The apparent protein digestibility of mulberry in tilapia diets has been reported at 80% (Shati et al., 2022).

Tithonia diversifolia is known for its protein and antioxidant content, contributing to digestibility and strengthening of the immune system, with a protein digestibility of approximately 78% (Herrera et al., 2020). On the other hand, Arachis pintoi provides essential proteins and lipids, improving the overall nutritional quality of the diet. Its apparent protein digestibility is around 75% in tilapia (ÇetiNkaya et al., 2020). Plukenetia vaolubilis, contributes with antioxidant and bioactive compounds that can improve nutrient absorption and intestinal health, although more studies are needed to determine its specific digestibility in tilapia (Lemus-Conejo et al., 2024).

Coffea arabica cacota and Theobroma cacao are rich sources of dietary fiber and antioxidants, although they have a low apparent fiber digestibility (40-50%), their inclusion at low levels promotes intestinal health and acts as a prebiotic (Vázquez-Sánchez et al., 2018; Soares & Oliveira, 2022). Arracacia macrorrhiza provides easily digestible carbohydrates and vitamins, with a positive impact on available energy and nutrient absorption. Its inclusion is strategic to balance fiber and improve the texture of the food (Sánchez-Bustos et al., 2021). Oryza sativa flour contributes to the energy intake and improves the palatability of the diet, with an apparent carbohydrate digestibility of more than 90% in tilapia (Sun et al., 2016).

On the other hand, the addition of other ingredients in the formulation of the experimental diet is essential, as in the case of vegetable oil, which provides essential fatty acids and improves the absorption of fat-soluble vitamins, reporting that lipid digestibility in tilapia generally exceeds 95% (Stoneham et al., 2018). Molasses provides quick energy and improves palatability, facilitating feed consumption, with a digestibility of 80-85%. Other compounds that should be added in small amounts are vitamins, minerals, salts, choline chloride, calcium carbonate, dicalcium phosphate, vitamin C and BHT (antioxidant). These elements are essential for bone health, immunity, and food stability, ensuring that the diet is complete and balanced, covering all micronutritional needs without significantly affecting total digestibility (Abdel-Fattah, 2020).

This formulated diet is balanced, with a focus on maximizing the digestibility of both proteins and carbohydrates, ensuring an adequate supply of essential nutrients for tilapia in the finishing phase. The combination of plant ingredients and supplements allows for efficient digestion, as well as providing optimal growth, aligned with sustainability practices in aquaculture. However, it is necessary to evaluate how differences in the nutritional content of these raw materials affect the productive variables of *O. niloticus*, so further studies in in-vivo animals are necessary to confirm the effect of dietary inclusion of these alternative foods on tilapia health and production.

Consideration of environmental benefits and challenges is essential when using these ingredients on a large scale, because arracacha and coffee production can have significant environmental impacts due to their need for extensive cultivation, which can result in deforestation or intensive water use (Giraldi-Díaz et al., 2018; Navarro-Niño et al., 2023). On the other hand, sacha inchi offers sustainable advantages due to its lower demand for resources, having the ability to grow in poor soils (Howlader, et al, 2023). Mulberry trees also have relatively low environmental impacts and can be a sustainable crop that could benefit aquaculture without compromising ecological integrity (Baciu et al., 2023). Assessing these factors is crucial to ensure effective and sustainable integration into fish production.

#### 4. CONCLUSION

The nutritional composition of various plant species and byproducts, considered as potential food sources for *Oreochromis niloticus*, shows significant variability in terms of nutrient content. These materials have relevant levels of protein, phosphorus and energy, suggesting that, properly combined with other sources, they could meet the specific nutritional requirements of tilapia.

This study offers valuable information for the formulation of balanced diets that not only meet the nutritional needs of *Oreochromis niloticus*, but also supports the sustainable development of aquaculture. The incorporation of these alternative ingredients into tilapia diets could reduce dependence on traditional feed sources, favoring both the diversification and sustainability of the aquaculture sector, especially in regions with limited access to conventional inputs. However, it is important to recognize that differences in the nutritional quality and digestibility of these materials can affect fish performance and health, highlighting the need for more research on this.

Regarding this, it is essential to carry out additional trials that validate the efficacy of these feed sources at different stages of the tilapia life cycle; detailed assessments of their impact on production performance and the quality of the final product will allow a better understanding of their viability as sustainable alternatives.

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AUTHOR'S CONTRIBUTION: José Luis Porras Vargas: Conceptualization, methodology design, research coordination. Adriana Patricia Muñoz Ramírez: Field data collection, research execution, data systematization. Carlos Eduardo Rodríguez Molano: Project supervision, funding acquisition, writing—review and editing, overall project administration. Néstor Julián Pulido Suárez: Statistical analysis, data visualization, validation of results, writing—review and editing. Laura Estefanía Niño Monroy: Literature review, research support, drafting of initial sections, critical revisions. Sergio Ulloa Torres: Experimental design, technical support, data curation, writing—review and editing. Valentina Rodríguez Montaña: proofreading and translation. Melisa Daniela Munevar Romero: Bibliographic management, supplementary material preparation, writing—review and editing, preparation of figures.

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